

## Commentary

# Why clinical trials of vitamin E and cardiovascular diseases may be fatally flawed. Commentary on “The Relationship Between Dose of Vitamin E and Suppression of Oxidative Stress in Humans”

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Many investigators have pondered the apparent paradox in the conflicting evidence about the cardiovascular benefits of vitamin E suggested by experimental and observational studies versus that reported from randomized clinical trials (RCT). Some appear to have already resolved the dilemma, concluding from these trials that it may be “past time for the scientific and public health communities to loosen their ties to a theory that lacks predictive ability for human disease” [1]. But has the “antioxidant theory” of disease prevention truly been tested in RCT? In this issue, Roberts et al. [2] characterize in patients with polygenic hypercholesterolemia the dose-response relationship between vitamin E and plasma F<sub>2</sub>-isoprostanes, a validated biomarker of lipid peroxidation. Their new data contribute importantly to a greater understanding of this paradox and should help to better inform the design of new human studies.

RCT of vitamin E targeting atherosclerosis and cardiovascular diseases (CVD) were warranted based on numerous studies indicating the bioactivity of this micronutrient *in vitro* and *in vivo* [3–5]. Studies in cell cultures have consistently demonstrated that vitamin E acts as an antioxidant and inhibits the proliferation of smooth muscle cells, reduces platelet adhesion and aggregation, and prevents monocyte-endothelial interactions. Supplementation with vitamin E in animal models indicates it acts to reduce oxidative stress, inhibit atherosclerotic lesion formation, and slow aortic intimal thickening. In human studies, vitamin E supplementation has been shown to act as an antioxidant by reducing various biomarkers of both oxidative stress and inflammation as well

as reducing platelet adhesion and aggregation. However, it was the early observational data from prospective cohorts such as the Nurses’ Health Study and the Health Professionals’ Follow-Up Study suggesting a benefit of vitamin E supplements in the primary prevention of CVD that probably did the most to generate interest in undertaking large scale RCT with vitamin E.

These observational data suggested that regular use of commercially available vitamin E supplements, typically 100–400 IU of *all-rac*- $\alpha$ -tocopherol (synthetic *dl*- $\alpha$ -tocopherol), were associated with a reduced risk of CVD. Thus, many RCT utilized this form and dose range of vitamin E hypothesizing they were similarly effective in the secondary prevention of CVD. Results from this new report by Roberts et al. [2] suggest use of the more bioavailable *RRR*- $\alpha$ -tocopherol (natural *d*- $\alpha$ -tocopherol) at doses 4–8-fold greater than those used in most RCT are required to obtain an effective reduction of oxidative stress. Regrettably, few other investigators have paid such close attention to the relationship between dose and pharmacokinetics of antioxidants and biomarkers of oxidative stress. Interestingly, Levine et al. [6] did examine these relationships for vitamin C but found no effect on plasma or urinary isoprostanes. Recently, the Women’s Antioxidant Cardiovascular Study, a RCT testing vitamin C (500 mg) daily alone or in combination with *RRR*- $\alpha$ -tocopherol (600 IU every other day) and  $\beta$ -carotene (50 mg every other day) found no benefit of these supplements on CVD outcomes in women at high risk or with a history of the disease [7]. However, it is worth noting this trial did find an 11 percent reduction ( $P=0.04$ ) in CVD morbidity and mortality in women with a history of the disease and taking vitamin E. Similarly, the Women’s Health Study, a RCT of primary prevention using

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this same dose and regimen of vitamin E, reported a 24 percent reduction in cardiovascular death ( $P=0.03$ ) though not in other CVD events [8].

Nonetheless, these and most other RCT of vitamin E have failed to determine whether the treatment intervention increased body or plasma  $\alpha$ -tocopherol status or affected any *in vivo* parameter of oxidative stress or inflammation or other biomarkers of cardiovascular function. Further, no RCT have employed cut-off values of vitamin E intake or status as inclusion criteria for enrollment eligibility. It is not evident that simply taking a vitamin E supplement reliably increases plasma  $\alpha$ -tocopherol. Not only does the form of vitamin E significantly affect its relative bioavailability, e.g., *all-rac*- vs. *RRR*- $\alpha$ -tocopherol, but also consumption absent the presence of an adequate amount of dietary fat may entirely prevent its absorption [9]. Thus, apparent compliance to the treatment (e.g., as assessed by pill count) may not indicate a change in blood and tissue concentrations of vitamin E. Even when the effort in RCT has been made to measure vitamin E status or oxidative stress, it is always restricted to selected subgroups, reducing the power of the analysis. As Roberts et al. [2] point out, the null outcome of the Primary Prevention Project RCT was reflected in the absence of a difference in urinary isoprostanes between the treatment (300 mg/d *all-rac*- $\alpha$ -tocopherol for 3 y) and control groups [10]. Absent evidence of significant changes in vitamin E and oxidative stress status, the antioxidant hypothesis is not being tested!

Assessing oxidative stress remains a daunting challenge, as its definition, an imbalance between the concentration of reactive oxygen species and the antioxidative defense mechanisms, is more conceptual than operational in nature. While dietary antioxidants are defined by their ability to decrease the adverse effects of reactive oxygen and nitrogen species [11], there is little agreement about which biomolecules serve as the best measure of oxidative stress. Despite the implication by Roberts et al. [2], measures of lipid peroxidation do not represent universal oxidative stress as there is no clear correlation between them and oxidation products of nucleic acids, proteins, or other cell constituents [12]. It is also important to appreciate the limitations associated with systemic biomarkers of oxidative stress in blood and urine when one principal site of action is the cellular milieu. Thus, we must be cautious in extrapolating the dose of vitamin E to all of its possible antioxidant actions as well as those mediated via other mechanisms, such as cell signaling. Nonetheless, the involvement of lipid peroxidation in atherogenesis indicates that isoprostanes are at least one important biomarker of oxidative stress in CVD.

Even with isoprostanes as a validated biomarker of lipid peroxidation, we are not yet able to compare changes in their concentration to physiological or pathological outcomes. While Roberts et al. [2] suggest the 49% reduction in plasma  $F_2$ -isoprostanes indicates “the antioxidant potency of vitamin E *in vivo* is not great,” this change may have a significant effect on cellular redox environment and function. The fact that isoprostanes are always present *in vivo* suggests not only a “normal” but even desirable range of this metabolite. Indeed,

some production of reactive oxygen species appears necessary for normal physiological function, e.g., signal transduction via modulation of kinases or phosphatases and transcription factor activation leading to cell growth, proliferation, and apoptosis. Thus, we need to appreciate a hierarchical model of oxidative stress with outcomes ranging from physiological to pathological. Further, given its dynamic nature, we would postulate no single constituent of the antioxidant defense network would be able to produce a maximum possible reduction in isoprostanes. Indeed, other antioxidants, including  $\alpha$ -lipoic acid, S-allyl-cysteine, and isoflavones, have been shown to contribute to reductions in isoprostanes.

Results from Roberts et al. [2] underscore the need to include appropriate assessment of the treatment (and its dose) and relevant biomarkers of oxidative stress in RCT of dietary antioxidants. This is particularly important because, as with all nutrient interventions, the controls are not at all a true placebo or “non-exposed” group but rather have a lifelong intake of antioxidant nutrients. While the dose-response data provided here from patients with polygenic hypercholesterolemia is informative, it may not extrapolate directly to other groups at risk of CVD, e.g., diabetics, elderly, and individuals with obesity or a history of smoking; therefore, similar studies with these cohorts are necessary. It is interesting to consider whether the hypercholesterolemic patients in this study had a higher concentration of substrate for isoprostane production such that correction of their results for arachidonic acid or total lipids would be necessary to distinguish the effect of fatty acid composition or content versus that of lipid peroxidation *per se* on isoprostane production. New dose-response data must also be generated when vitamin E is used in combination with other antioxidants. Further, especially in studies of secondary prevention with its concomitant polypharmacy and our growing knowledge of the modulation by vitamin E of xenobiotic metabolism [13], drug-nutrient interactions should be, but so far have never been, examined as a confounding factor in these RCT.

In contrast to suggestions from recent meta-analyses that supplemental vitamin E may induce adverse effects by acting as a pro-oxidant [14,15], Roberts et al. [2] show clearly that even at 3,200 IU/d, vitamin E works as an antioxidant. However, confusingly, they indicate “long term treatment with high dose vitamin E cannot be justified at this time.” Yet the Food and Nutrition Board [11] specifically notes “clinical trials of doses of  $\alpha$ -tocopherol above the UL should not be discouraged” so that important new information about both efficacy and safety can be obtained. Indeed, the results of this study suggest the paradox of vitamin E in CVD might be resolved by studies initiated at earlier ages, of longer durations, and using higher doses and more bioavailable forms of  $\alpha$ -tocopherol. In the near future, we may be able as well to include nutrigenomic parameters as eligibility criteria in new RCT to better identify likely “responders” to treatment. While final recommendations regarding the use of vitamin E for primary prevention of CVD should ultimately be based upon the totality of scientific evidence derived from experimental and observational studies as well as RCT, the latter must be designed and evaluated for

adequate dose and duration if they are to test the antioxidant hypothesis.

## References

- [1] Greenberg, E. R. Vitamin E supplements: Good in theory, but is the theory good? *Ann. Intern. Med.* **142**:75–76; 2005.
- [2] Roberts, L. J., II; Oates, J. A.; Linton, M. F.; Fazio, S.; Meador, B. P.; Gross, M. D.; Shyr, U.; Morrow, J. D. The relationship between dose of vitamin E and suppression of oxidative stress in humans. *Free Radic. Biol. Med.* **43**:1388–1393; 2007.
- [3] Sing, U.; Devaraj, S.; Jialal, I. Vitamin E, oxidative stress, and inflammation. *Ann. Rev. Nutr.* **25**:151–174; 2005.
- [4] Knekt, P.; Ritz, J.; Pereira, M. A.; O'Reilly, E. J.; Augustsson, K.; Fraser, G. E.; Goldbourt, U.; Heitmann, B. L.; Hallmans, G.; Liu, S.; Pietinen, P.; Spiegelman, D.; Stevens, J.; Virtamo, J.; Willett, W. C.; Rimm, E. B.; Ascherio, A. Antioxidant vitamins and coronary heart disease risk: a pooled analysis of 9 cohorts. *Am. J. Clin. Nutr.* **80**:1508–1520; 2004.
- [5] Traber, M. G. Heart disease and single-vitamin supplementation. *Am. J. Clin. Nutr.* **85**:293S–299S; 2007.
- [6] Levine, M.; Wang, Y.; Padayatty, S. J.; Morrow, J. A new recommended dietary allowance of vitamin C for healthy young women. *Proc. Natl. Acad. Sci. USA* **98**:9842–9846; 2001.
- [7] Cook, N. R.; Albert, C. M.; Gaziano, J. M.; Zaharris, E.; MacFadyen, J.; Danielson, E.; Buring, J. E.; Manson, J. E. A randomized factorial trial of vitamins C and E and beta carotene in the secondary prevention of cardiovascular events in women: Results from the Women's Antioxidant Cardiovascular Study. *Arch. Intern. Med.* **167**:1610–1618; 2007.
- [8] Lee, I.-M.; Cook, N. R.; Gaziano, J. M.; Gordon, D.; Ridker, P. M.; Manson, J. E.; Hennekens, C. H.; Buring, J. E. Vitamin E in the primary prevention of cardiovascular disease and cancer. The Women's Health Study: A randomized controlled trial. *JAMA* **294**:56–65; 2005.
- [9] Leonard, S. W.; Good, C. K.; Gugger, E. T.; Traber, M. B. Vitamin E bioavailability from fortified breakfast cereal is greater than that from encapsulated supplements. *Am. J. Clin. Nutr.* **79**:86–92; 2004.
- [10] Chiabrando, C.; Avanzini, F.; Rivalta, C.; Colombo, F.; Fanelli, R.; Palumbo, G.; Roncaglioni, M. C. PPP Collaborative Group on the antioxidant effect of vitamin E. Long-term vitamin E supplementation fails to reduce lipid peroxidation in people at cardiovascular risk: analysis of underlying factors. *Curr. Control Trials Cardiovasc. Med.* **19**:5; 2002.
- [11] Food and Nutrition Board, Institute of Medicine. Vitamin E. In: *Dietary reference intakes for vitamin C, vitamin E, selenium, and carotenoids*. Washington, DC: Natl. Acad. Press; 2000:186–283.
- [12] Dotan, Y.; Lichtenberg, D.; Pinchuk, I. Lipid peroxidation cannot be used as a universal criterion of oxidative stress. *Prog. Lipid Res.* **43**:200–227; 2004.
- [13] Mustacich, D. J.; Leonard, S. W.; Devereaux, M. W.; Sokol, R. J.; Traber, M. G.  $\alpha$ -Tocopherol regulation of hepatic cytochrome P450s and ABC transporters. *Free Radic. Biol. Med.* **41**:1069–1078; 2006.
- [14] Miller, E. R.; Pastor-Barriuso, R.; Dalal, D.; Riemersma, R. A.; Appel, L. J.; Guallar, E. Meta-analysis: high-dosage vitamin E supplementation may increase all-cause mortality. *Ann. Intern. Med.* **142**:37–46; 2005.
- [15] Bjelakovic, G.; Nikolova, D.; Gluud, L. L.; Simonetti, R. G.; Gluud, C. Mortality in randomized trials of antioxidant supplements for primary and secondary prevention. *JAMA* **297**:842–857; 2007.